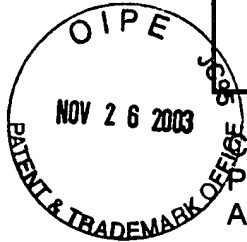
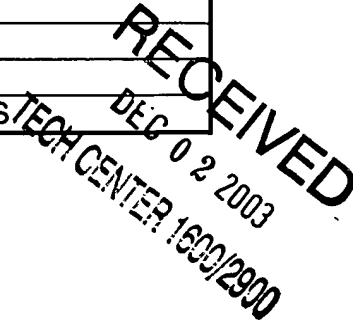


| | | |
|---|----------------|------------------|
| AMENDMENT TO EX PARTE QUAYLE ACTION AND STATEMENT IN RESPONSE TO SEQUENCE LISTING REQUIREMENTS | Application # | 09/494,297 |
| | Confirmation # | 3244 |
| | Filing Date | January 31, 2000 |
| | First Inventor | PODBIELSKI |
| | Art Unit | 1645 |
| | Examiner | Minnifield |
| | Docket # | P06628US0/BAS |



Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450



S I R:

In response to the *Ex Parte Quayle* Office Action dated August 28, 2003
2003, please amend the above identified application as follows.

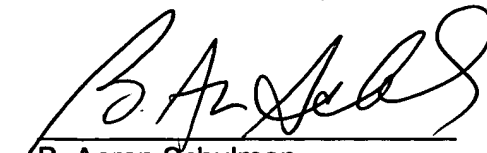
Please insert the attached Sequence Listing starting at Page 56 of the specification and substitute this sequence listing for any prior sequence listing in the application. **Applicants herein state that the attached sequence listing is identical to the computer readable form attached hereto and that the sequence listing adds no new matter to the application.**

Amendments to the Specification are reflected in the replacement paragraphs provided herewith in **Attachment A**.

Remarks to this Amendment are provided herewith in **Attachment B**.

In light of the above amendments and remarks included herein, the present application has now been placed in condition for allowance.

Respectfully submitted,
LARSON & TAYLOR, PLC


B. Aaron Schulman
Registration No. 31877

November 26, 2003

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ATTACHMENT A
Amended Paragraphs

At the following locations, please insert the following amended paragraphs.

Please amend the paragraph at page 6, lines 9-20 as follows.

Figure 1 is a schematic representation of a comparison of the *nra* (SEQ ID NO:5)/*rofA*-associated portions of group A streptococcal serotype M1, M6 and M49 strains. Results of pairwise comparisons of the deduced amino acid sequences of single ORF's are shown as percentage identity values between corresponding sequences. Sequence alignments were centered at the *nra* (SEQ ID NO:5)/*rofA* to *prtF/cpa* intergenic regions. All sequences are shown to scale. For designation of ORF's, see Table 1 hereinbelow. The M1 sequence was obtained from the GAS sequencing project (Roe et al., 1997), and the M6 sequence was taken from Hanski et al. (1992) and Fogg et al. (1994). The inserted box contains the comparison of the deduced *Nra* and *RofA* amino acid sequences. "." marks identical amino acid positions; "-" marks gaps that were introduced into the *RofA* sequence to maximize alignment. The underlined sequence marks the potential helix-turn-helix identified by Fogg et al. (1997).

Please amend the paragraph at page 45, lines 13-21 as follows.

Plasmid pFW11 was used for insertional mutagenesis as described by Podbielski et al. (1996c). Plasmid pFW11 multiple cloning site (MCS) 1. The

luciferase (*luc*) box was amplified by PCR using plasmid pUSL2/5 (Gräfe *et al.*, 1996) as template and oligonucleotides lucFor (5'GACGATCTCGAGGAGGTAAATGAAGACGCCAAAAAC-3') (SEQ ID NO:31) and lucRev (5'GACGATAAGCTTTTACAATTTGGACTTTCCG-3') (SEQ ID NO:32) as primers. The luciferase box contained an optimized Shine-Dalgarno sequence as well as the *luc* start and stop codons. Cloning of GAS genomic fragments into MCS1 of pFW11-luc followed the protocol outlined by Podbielski *et al.* (1996c).

Please delete Table 4 at page 43 and insert new Table 43 attached.

TABLE 4. List of oligonucleotides used in this work.

| Designation | Sequence (5' to 3') | Sequence ID. No. | Position Numbers | Reference |
|--------------|--------------------------|------------------|------------------|---------------------------|
| A. | | | | |
| nra FOR | ATTTTCTCATGTTGCTA | SEQ ID NO:6 | 6474-6492 | This study |
| nra REV | GTTTGAATGGTTAATTG | SEQ ID NO:7 | 7308-7290 | This study |
| rofA FOR | GCCAAATACTGAGTAGC | SEQ ID NO:8 | 141-158 | Fogg et al. (1994) |
| rofA REV | GGCTTTTGCTCTTTAGGT | SEQ ID NO:9 | 995-977 | Fogg et al. (1994) |
| cpa FOR | AGTTCACAAAGTTGCTACTG | SEQ ID NO:10 | 3435-3454 | This study |
| cpa REV | AAATAATAGATAGCAAGCTG | SEQ ID NO:11 | 3727-3708 | This study |
| prtF FOR | ATTAATGCCAGAGTTAGATG | SEQ ID NO:12 | 1414-1433 | Hanski and Caparon (1992) |
| prtF REV | CGATTCTCTCCACTTTG | SEQ ID NO:13 | 2259-2242 | Hanski and Caparon (1992) |
| prtF2 FOR | TACTCTGTAAAGAAAGTAACGTG | SEQ ID NO:14 | 2260-2281 | Jaffe et al. (1996) |
| prtF2 REV | CTCAGAGTCACCTTTCTGG | SEQ ID NO:15 | 3168-3151 | Jaffe et al. (1996) |
| nifR3 FOR | GGATTTTGCCTACTACTTA | SEQ ID NO:16 | 8443-8461 | This study |
| nifR3 REV | GTGGAATATCTAAACAGAC | SEQ ID NO:17 | 9313-9294 | This study |
| B. | | | | |
| nra-ins FOR | TTTTATTGGAGACTAGAGTTTA | SEQ ID NO:18 | 6325-6347 | This study |
| nra-ins REV | AGCAAGCCACTGATTTAC | SEQ ID NO:19 | 7481-7464 | This study |
| cpa-ins FOR | TGCAAAAGAGGGATAAAAC | SEQ ID NO:20 | 5932-5914 | This study |
| cpa-ins REV | GAAGCAGTAGACAACCTTGTC | SEQ ID NO:21 | 4707-4726 | This study |
| nraLuc FOR1 | TAACTAAAGTAGCTTAGCA | SEQ ID NO:22 | 5953-5972 | This study |
| nraLuc FOR5 | ATGGAACGTCATCACAAC | SEQ ID NO:23 | 6688-6705 | This study |
| nraLuc REV1 | CAGATACCTAAAAATAAACG | SEQ ID NO:24 | 7930-7911 | This study |
| cpa-pMAL FOR | GCTGAAGAACAAATCAGTACCA | SEQ ID NO:25 | 5798-5778 | This study |
| cpa-pMAL REV | TTAGTCATTTTTTAACCCCTTACG | SEQ ID NO:26 | 3705-3728 | This study |
| C. | | | | |
| RT-nra FOR | CTTTTACTATTAAAGAGATGA | SEQ ID NO:27 | 7669-7690 | This study |
| RT-nra REV | CTCGTTTAGAAAATCTTG | SEQ ID NO:28 | 7886-7869 | This study |
| RT-orf5 FOR | AAAATAATTAAATCAATAGCA | SEQ ID NO:29 | 8030-8050 | This study |
| RT-orf5 REV | CCACAGAGATAATGTGT | SEQ ID NO:30 | 8258-8241 | This study |

Oligonucleotides were used as primers to PCR amplify probes for Southern and Northern blot hybridizations (A), genomic fragments for cloning into pFW11, pFW11-luc or pMAL-c2 plasmids (B) and primers for RT-PCR to detect nra and orf5-specific transcripts (C). Primer pairs nra-ins FOR/REV, cpa-ins FOR/REV, nraLuc FOR/REV and cpa-pMAL FOR/REV were 5' extended with SphI/SpeI. NheI/BamHI and BAMHI/PstI sites, respectively, to facilitate forced cloning of the resulting PCR products. The nucleotide position numbers refer to the GAS nra genomic sequence as submitted to GenBank or the cited publications.